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Plant Protoplast Agglutination by Lectins

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ABSTRACT

Concanavalin A, soybean (*Glycine max L.*) lectin, castor bean (*Ricinus communis* L.) lectin, and peanut (*Arachis hypogaea* L.) lectin were able to agglutinate protoplasts prepared from a variety of plant species. The seven other lectins tried were unable to agglutinate those protoplasts tested. Protoplasts prepared from 11 species were used. The lectins examined were not able to differentiate among protoplasts of different species.

Recently there has been some interest in the effects of plant lectins on plant cell systems. *Phaseolus vulgaris* lectin was shown to have a mitogenic effect on tomato callus cells (13) and barley and pea root tip cells (17). Soybean lectin was mitogenic for soybean callus cells (9). PHA[2] and Con A both stimulate *Lilium* pollen germination (18). Golynskaya *et al.* (7) found hemagglutinins in *Primula* stigmata which also effected pollen tube growth. Pretreatment of *Gladiolus* stigmata with Con A prevented compatible pollen tubes from penetrating (10). There is also considerable evidence that the specificity of host-symbiont interactions is determined by lectin binding (1, 3, 4, 8, 15, 21).

Gamborg and Miller (5) suggested that lectins may be useful for protoplast aggregation which in turn would facilitate fusion. Since then Glimelius *et al.* (6) reported the agglutination of carrot culture cell protoplasts by Con A. They observed inhibition of the agglutination by methyl-α-D-glucoside and a great reduction of agglutination using glutaraldehyde-fixed protoplasts. Low temperature (4 C) limited agglutination and higher cell densities favored higher agglutination percentages. Burgess and Linstead (2) confirmed the ability of Con A to interact with protoplast surfaces. Using Con A conjugated with colloidal gold they demonstrated both specific binding (inhibited by 0.1 M α-methyl mannoside) to plasmalemma of *Vitis vinifera* culture and tobacco leaf protoplasts, and also nonspecific binding to amorphous material near the plasmalemma. The ability of plant lectins to interact with plasmalemma may be of significance to their physiological role. Ruesink (16) and Williamson *et al.* (19, 20) have also used labeled Con A as a protoplast membrane marker.

There has been no reported examination of any of the many other plant lectins for their effects on protoplasts. This report details some observations with Con A as well as 10 other lectins on some of 11 species of protoplast. If lectins were capable of differentiating among species of protoplast then it may be possible to use them to sort mixtures of protoplasts into subpopulations. This could be valuable for separating fusion hybrid protoplasts from parentals in the fusion mixtures of somatic hybridization.

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[2] Abbreviations: PHA: *Phaseolus vulgaris* lectin; Con A: concanavalin A; SBA: soybean lectin; WGA: wheat germ lectin; CBA II: castor bean type II lectin; FDA: fluorescein diacetate.

MATERIALS AND METHODS

Protoplast Isolation. Protoplasts were prepared from the various species with the procedures, enzymes, and osmotica as detailed in Larkin (12). The most usual enzyme incubation osmoticum was OSI (0.5 M mannitol, 150 mg/l $CaCl_2 \cdot 2H_2O$, 250 mg/l $MgSO_4 \cdot 7H_2O$, 100 mg/l KNO_3, 27.2 mg/l KH_2PO_4, 2.5 mg/l $Fe_2(SO_4)_3 \cdot 6H_2O$, 0.16 mg/l KI, 0.025 mg/l $CuSO_4 \cdot 5H_2O$). The cleaned protoplasts were always finally suspended in OSI (pH 7.2) before use. Viability was determined by the fluorescein diacetate technique (11) and only viable preparations were used.

Lectins. Soybean lectin and wheat germ lectin (WGA) were obtained from Pharmacia Fine Chemicals (Sweden) where they had been purified by affinity chromatography. Con A, *Ulex europaeus* (anti-H) lectin, *Dolichos biflorus* lectin, and *Phaseolus vulgaris* cv. red kidney lectin (PHA-M) were obtained from Calbiochem. PHA-M was prepared as a 40 to 80% saturation $(NH_4)_2SO_4$ cut of the initial seed extract. Castor bean type II lectin (CBA-II) and *Bandeiraea simplicifolia* lectin were obtained from Sigma Chemical Company and both were reported as consisting of one major electrophoretic protein band with trace bands.

Peanut lectin was extracted by a modification of the method of Lotan *et al.* (14). Peanuts (200 g, uncooked) were milled, defatted with 500 ml of petroleum ether, and air-dried. A suspension in 0.9% (w/v) NaCl was stirred for 3 hr and the insoluble matter removed twice by centrifugation. $(NH_4)_2SO_4$ was added to 60% (w/v) saturation and the centrifuged pellet redissolved in 110 ml of 0.9% (w/v) NaCl and dialyzed against 0.9% (w/v) NaCl for 24 hr with one change of saline. One sample was also dialyzed against OSI (pH 7.2) for 24 hr.

Vicia faba beans (40 g) were milled, defatted with 300 ml of petroleum ether, and dried in air. The flour was suspended and stirred for 1 hr in 200 ml of 0.9% (w/v) NaCl. The centrifuged sediment was discarded. The supernatant was fractionated by $(NH_4)_2SO_4$ precipitation into the 0 to 48%, 48 to 65%, 65 to 80% and 80 to 100% (w/v) saturation precipitates. These centrifuged pellets were redissolved in 40, 20, 20, and 25 ml of 0.9% NaCl, respectively. Each fraction was dialyzed against 0.9% (w/v) NaCl for 66 hr with one change of saline. On the basis of preliminary lectin specificity determinations it was decided to use only the 65 to 80 and 80 to 100% fractions for further experimentation. A sample of each of the 65 to 80 and 80 to 100% fractions was dialyzed against OSI (pH 7.2) for 24 hr.

All lectin solutions used with protoplasts were made up in OSI (pH 7.2) osmoticum to avoid protoplast damage.

Agglutination Tests. Agglutination was assessed by incubating protoplasts (10^6–10^7/ml) in test lectin solutions. Drops of test suspensions were placed in plastic Petri dishes at 22 to 27 C for 20 to 40 min with occasional rocking. Both lectins and protoplasts were in OSI (pH 7.2) osmoticum. Particular care was taken in the inhibition experiments to ensure that the osmolality was kept constant. As the concentration of test glycoside or sugar was varied, the concentration of mannitol (the major component of OSI) was varied to maintain a total concentration of sugars of 0.5

TABLE I. The interaction of plant protoplasts with lectins

Protoplasts (10^6–10^7/ml) were incubated in drops of test lectins in OSI (pH 7.2) for 20–40 min. Agglutination was assessed with microscopic examination on a subjective scale.

lectin	final conc. (mg/ml)	protoplasts: Nicotiana tabacum leaf	Nicotiana tomentosa leaf	Nicotiana miersii leaf	Nicotiana alata leaf	Petunia hybrida leaf	Vicia faba leaf	Daucus carota culture	Triticum aestivum leaf	Avena sativa leaf	Bromus inermis leaf	Zea mays leaf
SBA	0.5	++++ ‡	++++	n.a.	n.a.	++++	++++	+++	++++	++++	++++	n.a.
Con A	0.5	++++	+++	n.a.	n.a.	++++	+++	+++	+++	++++	n.a.	n.a.
WGA	0.5	-	-	-	n.a.	-	-	-	n.a.	-	n.a.	n.a.
Ulex	0.5	-	-	-	n.a.	-	-	n.a.	n.a.	-	n.a.	n.a.
PHA-M	0.5	-	-	n.a.	n.a.	-	-	n.a.	n.a.	-	n.a.	n.a.
CBA-II	0.5	++++	*	n.a.	+++	++++	+++	+++	++++	n.a.	n.a.	n.a.
Bandeiraea	0.05	-	-	-	n.a.	-	-	n.a.	n.a.	n.a.	n.a.	n.a.
Dolichos	0.5	n.a.	n.a.	n.a.	n.a.	n.a.	-	-	n.a.	n.a.	n.a.	-
Vicia extract 1†	½ dilution	-	-	-	n.a.	-	-	n.a.	n.a.	n.a.	n.a.	n.a.
Vicia extract 2†	½ dilution	-	-	-	n.a.	-	-	n.a.	n.a.	n.a.	n.a.	n.a.
Arachis extract	½ dilution	++++	++++	++++	n.a.	++++	+++	n.a.	+++	n.a.	n.a.	n.a.

† Vicia faba seed extracts 1 and 2 are the 65–80% and 80–100% $(NH_4)_2SO_4$ saturation fractions respectively

‡ ++++ = 75–100% agglutination; +++ = 50–75%; - = negligible; n.a. = not ascertained

* = +++ when protoplasts <50μm diam.; - " " " >100μm "

Table II. Potency of SBA-induced agglutination

Agglutination was assessed after 20 min at 27 C in OSI (pH 7.2) with the concentrations of SBA as shown.

SBA concentration (mg/ml)	0.5	0.1	0.05	0.01	0.005
N. tabacum leaf protoplasts (5×10^6/ml)	++++*	+++	+	-	-

* ++++ = 75–100% agglutination; +++ = 50–75%; + = 25%; - = negligible agglutination

M. Agglutination was assessed on a subjective scale using a light microscope.

RESULTS

Table I records the agglutinability of 10 species of plant protoplasts by lectins from 10 sources. Only Con A, SBA, CBA-II, and peanut extract agglutinated protoplasts. Figure 1, a, b, c, and d illustrates the agglutination reaction. The only lectin to show any specificity with respect to different protoplasts was CBA-II. This lectin agglutinated all of the species attempted except some preparations of *Nicotiana tomentosa* protoplasts. In some preparations these had unusually large diameters (>100 μm) and when this was so, CBA-II could not agglutinate them. Con A, SBA, and peanut extract could agglutinate these large protoplasts. Other *N. tomentosa* preparations (even from the same plant 2 weeks later) had more normal diameters (< 50 μm) and these could be agglutinated by CBA-II. It is not known why the diameters varied so dramatically in different preparations.

Table II shows the potency of SBA for agglutinating *Nicotiana tabacum* leaf protoplasts. The end point of agglutination was 0.05 mg/ml.

The effect of fixation on agglutinability was also investigated. A sample of *Bromus inermis* leaf protoplasts was fixed in 3% (w/v) glutaraldehyde for 2 hr. These fixed protoplasts had 0% viability as judged by the fluorescein diacetate (FDA) method (11). A low degree of spontaneous aggregation (approximately 10%) existed in the fixed suspension and 0.5 mg/ml SBA could not significantly increase this level of agglutination. The unfixed control suspension (approximately 90% FDA-viable) agglutinated in 0.5 mg/ml SBA to 75 to 100% aggregation.

Inhibition studies were used as a preliminary investigation of lectin specificities. Table III shows the effect of methyl-α-D-glucoside and methyl-β-D-galactoside on tobacco protoplast agglutination by the effective lectins. Methyl-α-D-glucoside was strongly inhibitory to Con A-induced agglutination and methyl-α-D-galactoside was strongly inhibitory to SBA-induced agglutination. Methyl-β-D-galactoside was to a lesser degree also inhibitory to peanut-, Con A-, and CBA-II-induced agglutination.

DISCUSSION

Only 4 out of 11 plant lectins were able to agglutinate protoplasts. These 4 lectins could agglutinate all species of protoplast with which they were tested. The only exception was CBA-II which could not agglutinate larger diameter *N. tomentosa* protoplasts (>100 μm). In each case agglutination could be inhibited by methyl-α-D-glucoside and/or methyl-β-D-galactoside. This indi-

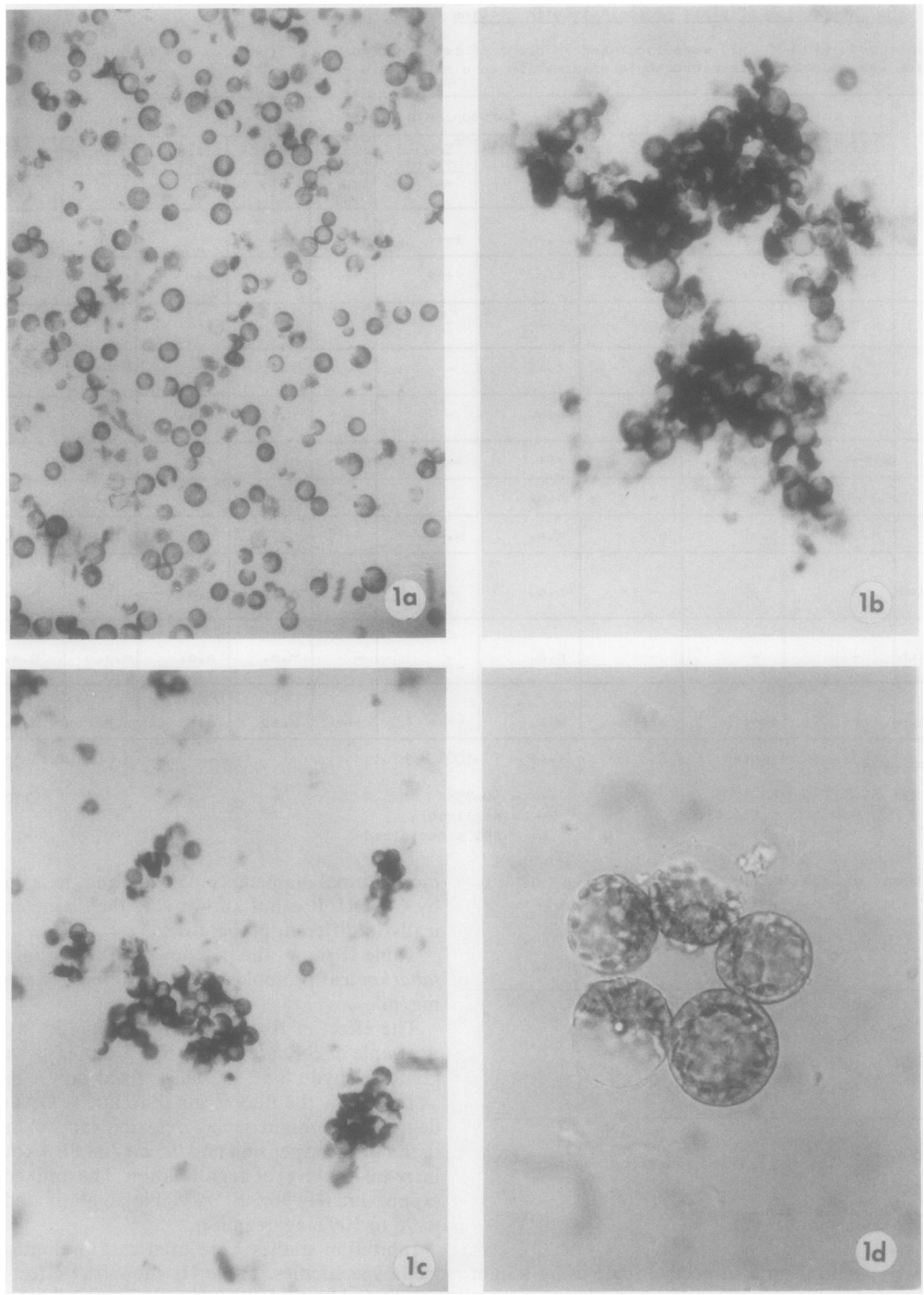

FIG. 1. *Triticum aestivum* mesophyll protoplasts suspended in OSI (pH 7.2) with test lectins. Agglutination was assessed after 30 min at 22 C with occasional rocking. a: Control in OSI (pH 7.2) (× 150); b: agglutination with 0.5 mg/ml SBA (× 200); c: agglutination with 0.5 mg/ml CBA-II (× 150); d: agglutination with ½ dilution peanut extract (× 600).

cated that the agglutinations were not due to some nonspecific interaction. It is possible that the nonagglutinating lectins are capable of binding to protoplast membrane receptors but that they are too small to span the distance between receptors on adjacent cells. This possibility could be examined using fluorescein isothiocyanate-labeled lectins and fluorescence microscopy. It is also possible that pretreatments of the protoplasts with enzymes such as trypsin or neuraminidase may expose receptors for the nonagglutinating lectins which may otherwise be masked.

It appears that plant lectins fail to differentiate between protoplasts of different species on the basis of agglutination. It remains to be shown whether these and other reported effects of lectins on plant cell systems correctly reflect their true physiological role. It must be considered unlikely that lectins will enable mixtures of different protoplast species to be sorted into the various subpopulations.

TABLE III. Glycoside inhibition of tobacco leaf protoplast agglutination.

N. tabacum protoplasts and the test lectins were incubated in drops of OSI (pH 7.2) modified by the addition of various concentrations of glycoside. Agglutination was assessed after 30 min.

GLYCOSIDE	Conc. † (mM)	LECTINS			
		Con A (0.5mg/ml)	Peanut lectin ($\frac{1}{2}$ dilution)	CBA-II (0.5mg/ml)	SBA (0.5mg/ml)
methyl-α-D-glucoside	0	+++*	++++	++++	++++
	10	-	++++	++++	++++
	30	-	++++	++++	+++
	60	-	++++	++++	+++
	100	-	++++	++++	+++
methyl-β-D-galactoside	0	+++	++++	++++	++++
	10	+++	++	++++	-
	30	++	++	+++	-
	60	+	++	++	-
	100	+	+	-	-

† The mannitol concentration of the OSI osmoticum was varied to compensate for the concentration of the added test glycoside. The protoplast density was approx. 5 x 10^6/ml.

*++++ = 75–100% agglutination; +++ = 50–75%; ++ = 25–50%; + = 25%; - = no agglutination.

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